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INFLAMMATORY BOWEL DISEASE

Analysis of the influence of OCTN1/2 variants within the IBD5 locus on disease susceptibility and growth indices in early onset inflammatory bowel disease

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Background and aims: The OCTN1 (SLC22A4 1672C→T) and OCTN2 (SLC22A5 –207G→C) variants within the IBD5 locus have been associated with susceptibility to adult onset Crohn's disease (CD), but their contribution in children has not been examined.

Methods: These OCTN1/2 variants and IBD5 marker single nucleotide polymorphisms (SNPs) (IGR2096a_1, IGR2198a_1, and IGR2230a_1) were examined in 299 Scottish children (200 with CD, 74 with ulcerative colitis (UC), and 25 with indeterminate colitis (IC)), together with 502 parents (for transmission disequilibrium testing) and 256 controls.

Results: All SNPs were in strong linkage disequilibrium ($D' > 0.94$). TDT analysis showed association of the OCTN1 variant with inflammatory bowel disease (IBD) ($p = 0.01$) and CD ($p = 0.04$). Allele frequencies of the OCTN1/2 variants were significantly higher in IBD/CD cases ($p < 0.04$). The homozygous mutant OCTN1/2 haplotype was increased in IBD (24.3% v 16.1%, $p = 0.02$) and UC (28.2% v 16.1%, $p = 0.02$) compared with controls. The OCTN1/2 variants were not independent of the background IBD5 risk haplotype in conferring disease susceptibility. Unifactorial analysis in CD patients showed that carriage of the TC haplotype was associated with lower weight, height, and BMI centile ($< 9^{\text{th}}$ centile) at diagnosis (weight: 87.9% v 67.3% ($p = 0.002$), odds ratio (OR) = 3.52 (95% confidence interval, 1.51 to 8.22); height: 84.1% v 68.4% ($p < 0.05$), OR = 2.44 (1.00 to 5.99); BMI: 79.6% v 61.1% ($p = 0.02$), OR = 2.49 (1.14 to 5.44)), and lower weight centile at follow up (87.5% v 64.6% ($p = 0.03$), OR = 3.83 (1.03 to 14.24)). Multifactorial binary logistic regression analysis confirmed association of the TC haplotype with lower weight centile at diagnosis ($p = 0.02$, OR = 3.41 (1.20 to 9.66)).

Conclusions: These data implicate variants within the IBD5 haplotype, as determinants of disease susceptibility and growth indices in early onset IBD. The OCTN1/2 variants remain potential positional candidate genes, but require further analysis.

The inflammatory bowel diseases (IBD) are chronic relapsing inflammatory conditions affecting the gastrointestinal tract, comprising Crohn's disease (CD), ulcerative colitis (UC), and indeterminate colitis (IC). The aetiology of IBD is unknown, although there is strong evidence for a gene–environment interaction.¹ Around 15–25% of cases will present in childhood.² The incidence of paediatric IBD in Scotland is higher than elsewhere in the United Kingdom and has increased threefold in the past 30 years.^{3–5}

IBD starting in childhood can have profound effects on a child's growth, pubertal development, and education. Growth failure is a common feature of childhood IBD, particularly in Crohn's disease.⁶ Several factors have been linked to growth failure in Crohn's disease including delay to diagnosis,⁷ jejunal inflammation,⁸ disease severity,⁹ age at diagnosis,¹⁰ and genotype.¹¹

Genome-wide scanning in IBD has identified various susceptibility loci, with two that are particularly noteworthy in early onset disease—IBD1 and IBD5.¹² The contribution of the NOD2/CARD15 gene to genetic susceptibility to Crohn's disease varies between populations. In Scotland and northern Europe this effect is significantly less strong than in many other paediatric and adult IBD populations.¹³

The IBD5 locus (5q31–33) was first identified in genome-wide scanning of North American Crohn's disease

patients.^{14–15} Rioux *et al* stratified the genetic data from the Canadian genome-wide scan by age, and showed that the highest LOD (log of odds) score was found in patients with Crohn's disease diagnosed under 16 years of age.¹⁴

In a detailed study of the IBD5 locus, Daly and colleagues reported strong linkage disequilibrium across the region, and derived a risk haplotype for Crohn's disease that as represented by 11 marker single nucleotide polymorphisms (SNPs) in separate haplotype blocks that spanned the whole 250 kb interval.¹⁶ Heterozygotes for the IBD5 risk haplotype had a twofold increased risk of Crohn's disease, and homozygotes a sixfold increase, but with no increased risk of ulcerative colitis.¹⁷ Several European studies have now replicated the association of IBD5 with susceptibility to adult onset Crohn's disease,^{18–21} and additionally one study has shown an association with ulcerative colitis.¹⁸ Genotype–phenotype studies in adult onset disease have shown association with perianal Crohn's disease²⁰ and earlier age of disease onset.¹⁹ IBD5 epistasis has been demonstrated with the IBD6 locus²² and with NOD2/CARD15, for both Crohn's disease¹⁹ and ulcerative colitis.^{18–23}

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; IC, indeterminate colitis; SNP, single nucleotide polymorphism; UC, ulcerative colitis

Two variants within the IBD5 interval have been suggested to be independently associated with Crohn's disease; variant alleles of the OCTN1 gene (SLC22A4 C/T, missense mutation) and OCTN2 (SLC22A5 -207 G/C, promoter mutation).²⁴ Both of these genes have been suggested to play a role in carnitine transport but critical expression and functional data in IBD patients are still awaited. In the initial publication from Peltekova and colleagues, the resulting two allele risk (TC) haplotype was independently associated with susceptibility to Crohn's disease when Crohn's disease patients and controls who were homozygous wild type for marker SNP IGR2078a_1 were compared.²⁴ Moreover, several subsequent adult studies have been unable to confirm that the OCTN1/2 effect is independent of the other potential determinants within the extended IBD5 haplotype.^{25, 26}

In this study we have analysed the contribution to disease susceptibility and phenotype of three markers on the IBD5 haplotype, together with the OCTN1/2 variants and the TC haplotype within a large homogenous paediatric IBD population. We have specifically examined whether the OCTN1/2 effect is independent of other determinants within the IBD5 locus. In addition our detailed phenotypic data have allowed us to examine the effect of these markers on growth indices.

METHODS

Patients

We recruited 299 patients with IBD diagnosed at less than 16 years of age from Scottish paediatric gastroenterology centres and from the Western General Hospital, Edinburgh. Two hundred patients had an established diagnosis of Crohn's disease, 74 ulcerative colitis, and 25 indeterminate colitis.

Parents and controls

We also enrolled 502 parents to construct family trios for transmission disequilibrium testing (TDT) (71% of the patients had complete family trios). DNA from 256 healthy adult controls was also available for case-control analysis.²⁶

Disease phenotype

Standardised criteria were used for IBD diagnosis.²⁷ A patient was categorised as having "indeterminate colitis" if definite evidence of chronic inflammatory bowel disease affecting the colon only was present, but the patient remained unclassifiable as either Crohn's disease or ulcerative colitis after considering all clinical, radiological, endoscopic, and pathological findings. The location and behaviour of Crohn's disease was assessed at the time of diagnosis and at two yearly intervals after diagnosis, categorised using the Vienna classification.²⁸ Disease location in Crohn's disease was also categorised and analysed according to disease location regardless of disease involvement elsewhere in the gastrointestinal tract, as previously described.¹³ The use of this comprehensive complementary classification system overcomes some of the recognised limitations of the Vienna classification, notably the difficulties of categorising patients with disease involving both the upper gastrointestinal tract and other sites.²⁹ In this classification, patients were diagnosed with Crohn's disease of the upper gastrointestinal tract (oesophagus, stomach, and duodenum) when biopsies from any of these sites confirmed the presence of epithelioid granulomas (not merely in the presence of chronic inflammation), or when features of macroscopic disease were present,³⁰⁻³² or both. Perianal disease was defined by the presence of anal fissures, perianal abscesses, fistulae, and perianal ulcers, consistent with recommendations of Lennard-Jones *et al.*²⁷ To allow comparison with the results published by other groups,^{20, 33} we also analysed data using the more limited definition of perianal disease applied by these investigators, who excluded anal fissures.

Table 1 Demographic variables at the time of diagnosis in the Crohn's disease patients included in the study

Sex (M/F)	121/79
Median age at diagnosis (y)	11.2 (IQR 8.6 to 13.0)
Current smoker	3 (2%)
Family history	68 (34%)
White (%)	195 (97%)
Location according to the Vienna classification²⁸	
Terminal ileum (L1)	9 (4.6%)
Colon (L2)	48 (24.4%)
Ileocolon (L3)	38 (19.2%)
Upper gastrointestinal (L4)	93 (47.2%)
None*	9 (4.6%)
Behaviour according to the Vienna classification²⁸	
Inflammatory (B1)	163 (82.7%)
Strictureing (B2)	7 (3.6%)
Penetrating (B3)	27 (13.7%)
Disease location†	
Oral	27/182 (14.8%)
Oesophageal	7/170 (4.1%)
Gastric antrum	49/168 (29.2%)
Gastric body	32/167 (19.1%)
Duodenal	26/167 (15.6%)
Jejunal	29/165 (17.6%)
Ileal	111/173 (64.2%)
Caecal	108/142 (76.0%)
Ascending	110/146 (75.3%)
Transverse	119/156 (76.3%)
Descending	125/167 (77.1%)
Sigmoid	137/174 (78.7%)
Rectal	139/182 (76.4%)
Perianal‡	80/190 (42.1%)
Perianals	20/190 (10.5%)
Anthropometry¶	
Weight <9th centile	71/185 (38.4%)
Weight <25th centile	104/185 (56.2%)
Height <9th centile	46/178 (25.8%)
Height <25th centile	83/178 (46.6%)
BMI <9th centile	66/178 (37.1%)
BMI <25th centile	92/178 (51.7%)
Mean weight z score (SD)	-0.68 (1.55)
Mean height z score (SD)	-0.48 (2.01)
Mean BMI z score (SD)	0.66 (1.60)
Associated diseases	
Asthma	58/198 (29.3%)
Eczema	68/198 (34.3%)
Hayfever	41/198 (20.7%)
All three diseases	15/198 (7.9%)

The phenotypic data at diagnosis are based on 197 Crohn's disease patients unless otherwise stated.

*These were patients who fulfilled the diagnostic criteria for Crohn's disease but whose disease location does not allow the use of the Vienna classification; all had perianal or oral Crohn's disease or both without evidence of disease elsewhere in the gastrointestinal tract.

†The presence or absence of evidence for Crohn's disease activity in each location was based on evidence from endoscopy, biopsy, or barium follow through and was independent of disease activity elsewhere in the gastrointestinal tract.

‡Perianal disease was defined by the presence of fissures, perianal ulcers, abscesses, or fistulae but not by the presence of skin tags alone.

§Perianal disease was defined by the presence perianal ulcers, abscesses, or fistulae but not by the presence of skin tags or fissures.

¶Based on centile plotted on UK Growth Chart (© Child Growth Foundation, 1996).

BMI, body mass index; IQR, interquartile range; y, years.

Data collection

Disease phenotype was determined using a combination of patient questionnaire, interview, and retrospective case note review, as previously described.¹³ Information collected included weight, height, and body mass index (BMI) values, which were plotted on a centile chart (© Child Growth Foundation, 1996) and an appropriate centile band allocated.

Table 2 Demographic variables at the time of diagnosis in the ulcerative colitis patients included in the study

Sex (M/F)	32/37
Median age at diagnosis (y)	10.7 (IQR 8.5 to 12.9)
Current smoker	1 (1.4%)
Family history	20 (29.0%)
White (%)	66 (95.6%)
Disease extent	
Pancolitis	36 (52.2%)
Extensive colitis*	13 (18.8%)
Left sided colitis†	16 (23.2%)
Proctitis	4 (5.8%)
Colectomy (%)‡	9/64 (14.1%)
Extraintestinal manifestations (%)	7/67 (10.4%)
Anthropometric measurements§	
Weight <9th centile	11/65 (16.9%)
Weight <25th centile	20/65 (30.8%)
Height <9th centile	9/59 (15.2%)
Height <25th centile	23/59 (39.0%)
BMI <9th centile	6/59 (10.2%)
BMI <25th centile	19/59 (15.2%)

The data refer to 69 patients at disease diagnosis unless otherwise stated.

*Inflammation beyond the splenic flexure excluding patients with pancolitis.

†Inflammation distal to the splenic flexure excluding patients with proctitis.

‡The number of patients who had had a colectomy by the time of last clinic follow up.

§Based on centile plotted on UK Growth Chart (© Child Growth Foundation, 1996).

BMI, body mass index; IQR, interquartile range; y, years.

Each patient had their height measured on a wall mounted stadiometer. Anthropometric data were also defined using z scores. A z score for a growth parameter indicates how far and in what direction that item deviates from its distribution's mean, expressed in units of its distribution's standard deviation. The demographics details of the patients with Crohn's disease and ulcerative colitis in the study are shown in tables 1 and 2, respectively.

Ethical approval was given for all participating centres. All families gave informed written consent before participation in the study.

Genotyping

Genomic DNA was extracted from blood using a modified salting-out technique,³⁴ and resuspended in 1×TE (10 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0)) at a final concentration of 100 ng/μl. Three marker SNPs on the IBD5 haplotype were typed IGR2096a_1, IGR2198a_1, and IGR2230a_1. The rs1050152 polymorphism of the OCTN1 gene (SCL22A4 exon 9 1672C→T) and the rs26313667 (SLC22A5 promoter, -207G→C) polymorphism of the OCTN2 gene were also typed. The primers used in the study are given in table 3.

The relative positions of these markers within the haplotype blocks described by Daly *et al* are shown in fig 1. All genotyping was carried out using the TaqMan system (©Biosystems). The three NOD2/CARD15 mutations (Leu1007InsC, G908R, and R702W) were genotyped as previously described.³⁵

Statistical analysis

Each of the five SNPs was analysed individually using allele frequencies and carriage rates for association with IBD, Crohn's disease, and ulcerative colitis. As the total number of patients with indeterminate colitis was small these data were included in IBD analysis overall and were not analysed as an individual disease group. TDT analysis was carried out using Transmit (version 2.5)³⁶ after the Pedcheck software program was used to exclude any potential cases of non-paternity or

Table 3 Primers used to examine each of the five single nucleotide polymorphisms involved in this study

IGR number	TaqMan primers
IGR2096	Forward: TCTGAGACAGGAGCCACTAGAG Reverse: CACAGCATCCAGAGTGATCCT
IGR2198	Forward: GGGTTGCATGAGCATTAAAGTTCAA Reverse: CCACATCAAGGATAAGACTGCTAAATACT
IGR2222	Forward: GCGGCTGGCCTTACATAGG Reverse: CCGCTCTGCCTGCCA
IGR2230	Forward: GCAGGCAGAACAGCCATACT Reverse: GGCCACAGAACTTCAATAAAGTAGGA
IGR3002	Forward: TTTACAGGTGCTTACAACAGAATG Reverse: TAGTCTGACTGTCTGATTGGAATC

genotyping error.³⁷ Genotype-phenotype relations were determined by χ^2 or Fisher's exact test using Minitab v.13 (Minitab Ltd, Coventry, UK). Multifactorial analysis was done using binary logistic regression analysis. Haplotypes were calculated using Haploview, v.3.2. All SNPs in patients and controls were in Hardy-Weinberg equilibrium. We calculated z scores using a program designed by Dr C Wright (Glasgow University) and values were compared by the Mann-Whitney test. Odds ratios (OR) and 95% confidence intervals (CI) are given.

RESULTS

Strong linkage disequilibrium was seen between the three IBD5 haplotype SNPs and the two OCTN variants (fig 2). The pairwise D' scores for all five markers were 0.94 or above.

Transmission disequilibrium testing analysis

These results are shown in table 4. The IGR2198a_1 allele was associated with susceptibility to IBD ($p = 0.02$). Homozygosity for the mutant IBD5 haplotype (patients who were homozygous mutants for all three IBD5 haplotype marker variants IGR2096a_1, IGR2198a_1, and IGR2230a_1) was associated with susceptibility to Crohn's disease ($p < 0.05$). The OCTN1 variant was associated with susceptibility to IBD and Crohn's disease ($p = 0.01$ and $p = 0.04$, respectively). The homozygous OCTN1/2 mutant haplotype was associated with susceptibility to IBD ($p = 0.01$).

Case-control analysis

Inflammatory bowel disease

The results are given in table 5. The allele frequencies were significantly higher in IBD patients for all three IBD5 SNPs and for the OCTN1/2 variants than in healthy controls ($p = 0.01$ to 0.04). Homozygosity rates for the IBD5 variant alleles IGR2096a_1 and IGR2198a_1, as well as homozygosity rates for the mutant TC haplotype in patients with IBD, were significantly higher than in healthy controls:

IGR2096a_1: 24.4% v 15.2% ($p = 0.008$), odds ratio (OR) = 1.79 (1.16 to 2.78);

IGR2198a_1: 22.8% v 15.2% ($p = 0.03$), OR = 1.64 (1.06 to 2.54);

mutant TC haplotype: 24.3% v 16.1% ($p = 0.02$), OR = 1.67 (1.08 to 2.58).

Crohn's disease

These results are shown in table 6. The frequencies of variant alleles in Crohn's disease patients compared with healthy controls were significantly higher for the variants:

IGR2096a_1: 49.2% v 42.0% ($p = 0.04$), OR = 1.34 (1.02 to 1.75);

IGR2198a_1: 47.7% v 41.0% ($p = 0.04$), OR = 1.31 (1.01 to 1.71);

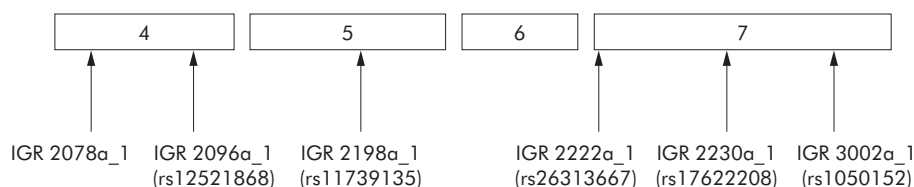


Figure 1 The relative position within the haplotype blocks of the IBD5 locus (as described by Daly *et al.*⁶) of the five single nucleotide polymorphisms (SNPs) within the haplotype examined in this study. Also shown is the marker SNP IGR 2078a_1 examined in the study by Peltekova *et al.*²⁴ The corresponding rs numbers are given below each SNP examined in this study. In Daly's original study, 11 separate haplotype blocks were described; the numbers shown here are the ones referred to in the original publication. IGR 2222a_1 refers to the OCTN2 variant (−207G→C) and IGR 3002a_1 refers to the OCTN1 variant (1672C→T).

OCTN2: 54.8% *v* 47.9% ($p = 0.04$), OR = 1.32 (1.01 to 1.72).

Homozygous carriage of mutant IGR2096a_1 was higher in Crohn's disease patients than in the controls, at 23.6% *v* 15.2% ($p = 0.03$), OR = 1.72 (1.06 to 2.79).

Ulcerative colitis

These results are shown in table 7. The frequency of the IGR2198a_1 variant allele was higher in the patients with ulcerative colitis than in the controls (50.7% *v* 41.0% ($p = 0.04$), OR = 1.48 (1.02 to 2.15)). Homozygosity for the variant alleles was higher in ulcerative colitis patients than controls, as follows:

IGR2096a_1: 27.9% *v* 15.2% ($p = 0.01$), OR = 2.16 (1.15 to 4.05);

IGR2198a_1: 26.8% *v* 15.2% ($p = 0.04$), OR = 1.92 (1.03 to 3.58).

Homozygous carriage of the mutant TC haplotype and of a combined IBD5 haplotype (homozygosity for all three IBD5 marker SNP variants) was also higher in patients with ulcerative colitis than in controls:

mutant TC haplotype: 28.2% *v* 16.1% ($p = 0.02$), OR = 2.04 (1.10 to 3.78);

combined IBD5 haplotype: 26.6% *v* 14.7% ($p = 0.02$), OR = 2.10 (1.09 to 4.05).

Association between the OCTN1/2 TC haplotype and disease susceptibility

There was no independent association between the TC haplotype and susceptibility to IBD, Crohn's disease, or ulcerative colitis in individuals lacking the extended IBD5

risk haplotype markers (IGR2198a_1, IGR2230a_1, and IGR2096a_1) (table 8). The data shown in table 8 do not show a significant differences between IBD, Crohn's disease, or ulcerative colitis compared with controls in the carriage of the variant alleles representing the TC haplotype on a background of having no IGR2198a_1 risk alleles. Thus for Crohn's disease only four of 47 patients carried the TC haplotype but no IGR2198a_1 variants, compared with 10 of 82 IGR2198a_1 variant negative controls ($p = 0.57$). The same lack of association was also seen with the TC haplotype if either of the two other variants (IGR2230a_1 and IGR2096a_1) was analysed similarly, or if all three IBD5 SNPs were combined (data available on request).

No independent effect was demonstrated if the OCTN variants 1 and 2 were considered individually rather than as the TC haplotype (data available on request).

Epistasis with NOD2/CARD15 mutations

There was no evidence of epistasis between IBD/CD patients with regard to the TC haplotype and patients carrying the common NOD2/CARD15 mutations (Leu1007InsC, G908R, and R702W) in NOD2/CARD15 carriers *v* non-carriers for IBD and Crohn's disease (70.3% *v* 76.8% ($p = 0.39$) and 71.0% *v* 76.9% ($p = 0.49$), respectively).

Genotype–phenotype analysis: unifactorial analysis

For clarity, the results presented in the genotype–phenotype analysis (table 9) describe associations with the TC haplotype only, with the exception of growth indices. The phenotypic associations with the TC haplotype generally reflected the

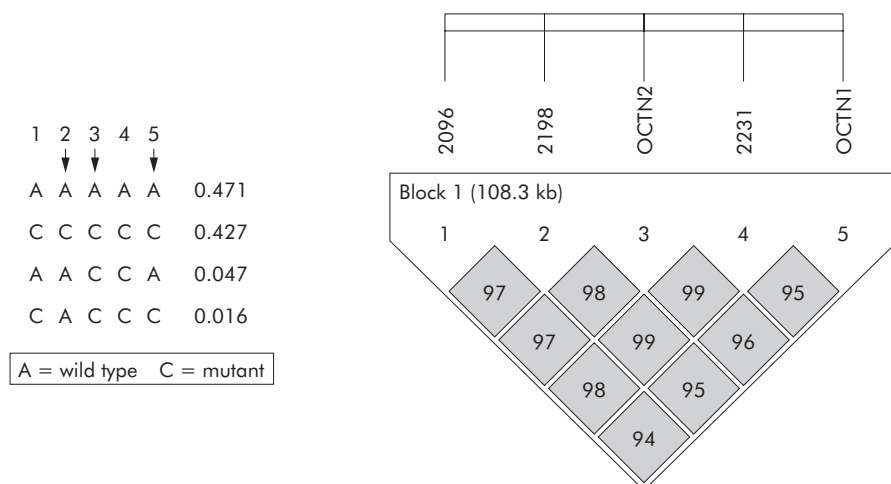


Figure 2 The D' scores of the five single nucleotide polymorphisms studied across the IBD5 haplotype and the common haplotype frequencies.

Table 4 Transmission disequilibrium testing results for IBD5 and OCTN variants in patients with inflammatory bowel disease

Mutation	IBD		CD		UC	
	χ^2	p Value	χ^2	p Value	χ^2	p Value
IGR2230a_1	2.42	0.12	0.87	0.35	2.00	0.16
IGR2198a_1	5.57	0.02	2.24	0.15	4.03	0.06
IGR2096a_1	2.34	0.16	1.11	0.32	1.36	0.24
IBD5 haplotype	10.59	0.05	10.13	<0.05	4.07	0.42
OCTN1	7.54	0.01	4.21	0.04	3.54	0.06
OCTN2	1.57	0.23	0.47	0.52	1.54	0.18
TC haplotype	12.92	0.01	6.59	0.11	7.72	0.21

The results were based on 293 patients with IBD, together with one or both parents (the numbers of informative trios were 271/197/74 for IBD/CD/UC, respectively). The results represented are for the allelic variants of the IBD5 marker single nucleotide polymorphisms IGR2096a_1, IGR2198a_1, and IGR2230a_1, the 1672C→T variant of the OCTN1 gene, and the -207G→C variant of the OCTN2 gene. The IBD5 haplotype in this table refers to the homozygosity for mutants of IGR2096a_1, IGR2198a_1, and IGR2230a_1 combined; the TC haplotype refers to homozygosity for the mutants of OCTN1/2 combined.

CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

genotype-phenotype results for all SNPs analysed across the IBD5 haplotype (all SNPs in close linkage disequilibrium). Supplementary information on the IBD5 alleles and OCTN variant alleles individually is available on request.

Growth indices

A comparison was made between lowest three (<9th) centile groupings and the remaining seven (>9th) centiles for weight, height, and BMI. Carriage of the TC haplotype was more common in patients with lower weight, height, and BMI than in those in higher centiles at diagnosis of Crohn's disease:

lower weight: 87.9% v 67.3% ($p = 0.002$), OR = 3.52 (1.51 to 8.22);

lower height: 84.1% v 68.4% ($p < 0.05$), OR = 2.44 (1.00 to 5.99);

lower BMI: 79.6% v 61.1% ($p = 0.02$), OR = 2.49 (1.14 to 5.44).

Analysis using median z scores comparing homozygous carriers of the TC haplotype with non-carriers confirmed a significant difference between the groups in BMI (-1.32 v -0.51 , $p = 0.03$) and for weight (-1.16 v -0.49 , $p = 0.05$) but not for height ($p = 0.89$).

At two year follow up the TC haplotype was more common in lower than in higher weight centiles, at 87.5% v 64.6% ($p = 0.03$), OR = 3.83 (1.03 to 14.24).

Carriage of the TC haplotype showed a significance difference for weight centile (<25th centile v >25th centile): 83.0% v 65.8% ($p = 0.01$), OR = 2.54 (1.23 to 5.23) at diagnosis; 88.9% v 58.5% ($p = 0.002$), OR = 5.68 (1.75 to 18.37) at the two year follow up.

Disease location

Homozygous carriage of the TC haplotype was associated with gastric antral Crohn's disease, when comparison was made with patients assessed and found to have no antral disease at diagnosis (36.7% v 14.2% ($p = 0.006$), OR = 3.51 (1.40 to 8.83)).

Asthma, eczema, hayfever, and atopy

Homozygosity for the TC haplotype was less often seen in patients with asthma than in those without (11.5% v 26.9% ($p = 0.02$), OR = 0.35 (0.14 to 0.90)); was less common in patients with hayfever than in those without (7.5% v 27.0% ($p = 0.009$), OR = 0.22 (0.06 to 0.75)); and was less common in patients who had all three atopic diseases combined

Table 5 Allele frequencies and carriage rates for variants in the IBD5 haplotype in patients with inflammatory bowel disease compared with healthy controls

	IBD patients (n = 299) v healthy controls (n = 256)	p Value	Odds ratio
Allelic frequency			
IGR2096a_1	49.6% v 42.0%	0.01	1.36 (1.07 to 1.73)
IGR2198a_1	48.4% v 41.0%	0.01	1.35 (1.07 to 1.72)
IGR2230a_1	53.8% v 47.5%	0.04	1.29 (1.01 to 1.64)
OCTN 1	50.0% v 42.9%	0.02	1.33 (1.05 to 1.69)
OCTN 2	54.5% v 47.9%	0.03	1.30 (1.03 to 1.66)
Homozygous carriage			
IGR2096a_1	24.4% v 15.2%	0.008	1.79 (1.16 to 2.78)
IGR2198a_1	22.8% v 15.2%	0.03	1.64 (1.06 to 2.54)
IGR2230a_1	27.0% v 21.2%	0.12	1.38 (0.92 to 2.05)
TC haplotype	24.3% v 16.1%	0.02	1.67 (1.08 to 2.58)
Combined IBD5 carriage	21.3% v 14.7%	0.06	1.57 (0.99 to 2.49)

Three marker single nucleotide polymorphisms (SNPs) (IGR2096a_1, IGR2198a_1, and IGR2230a_1) were used to represent the IBD5 haplotype together with two SNPs in the OCTN1 and 2 (1672C→T and -207G→C, respectively). All five SNPs were associated with susceptibility to inflammatory bowel disease (IBD). Homozygous carriage of IGR2096a_1, IGR2198a_1, and the TC haplotype were associated with susceptibility to IBD. The results for heterozygous carriage all failed to achieve significance (results not shown).

IBD, inflammatory bowel disease.

Table 6 Allele frequencies and carriage rates for variants in the IBD5 haplotype in Crohn's disease patients compared with healthy controls

	CD patients (n = 200) v healthy controls (n = 256)	p Value	Odds ratio
Allelic frequency			
IGR2096a_1	49.2% v 42.0%	0.04	1.34 (1.02 to 1.75)
IGR2198a_1	47.7% v 41.0%	0.04	1.31 (1.01 to 1.71)
IGR2230a_1	53.8% v 47.5%	0.06	1.29 (0.99 to 1.69)
OCTN 1	49.5% v 42.9%	0.05	1.30 (1.00 to 1.70)
OCTN 2	54.8% v 47.9%	0.04	1.32 (1.01 to 1.72)
Homozygous carriage			
IGR2096a_1	23.6% v 15.2%	0.03	1.72 (1.06 to 2.79)
IGR2198a_1	20.8% v 15.2%	0.12	1.46 (0.90 to 2.38)
IGR2230a_1	26.9% v 21.2%	0.16	1.37 (0.88 to 2.14)
TC haplotype	22.2% v 16.1%	0.11	1.48 (0.91 to 2.40)
Combined IBD5 haplotype	19.2% v 14.7%	0.42	1.38 (0.82 to 2.32)

Three marker single nucleotide polymorphisms (SNPs) (IGR2096a_1, IGR2198a_1, and IGR2230a_1) were used to represent the IBD5 haplotype together with two SNPs in the OCTN1 and 2 (1672C→T and -207G→C, respectively). The three SNPs IGR2096a_1, IGR2198a_1, and -207G→C were associated with susceptibility to Crohn's disease. Homozygous carriage of the IGR2096a_1 was also associated with susceptibility to Crohn's disease. The results for heterozygous carriage all failed to achieve significance (results not shown).
CD, Crohn's disease.

(asthma, eczema, and hayfever) than in those without (0% v 25.3% (p = 0.02)).

Disease behaviour and need for surgery

At Crohn's disease diagnosis and at the most recent follow up no association was seen between carriage of the TC haplotype and disease behaviour. During the period of analysis 26.9% of patients underwent abdominal surgery for Crohn's disease. Carriage of the TC haplotype was no higher in those patients needing surgery than those who did not (77.6% v 73.9% (p = 0.61)).

Age at diagnosis

The median age at diagnosis of carriers of the TC haplotype was 11.6 years (v 10.9 years for non-carriers (p = 0.12)) and for TC homozygotes it was 12.1 years (v 11.3 years in non-carriers (p = 0.06)).

Other phenotypic variables

No association was seen with the TC haplotype and family history of IBD, extraintestinal manifestations, granulomas, or

blood test abnormalities (full blood count, erythrocyte sedimentation rate, C reactive protein, or albumin concentration).

Ulcerative colitis

Carriers of the TC haplotype were more common in patients in lower height centiles at diagnosis (<25th centile v >25th centile: 85.7% v 55.9% (p = 0.02), OR = 4.74 (1.17 to 19.16)) and showed a trend towards significance for weight centile (83.3% v 59.5% (p = 0.07), OR = 3.40 (0.85 to 13.57)). Genotype-phenotype analysis in this group was limited by the small number of patients available for analysis (n = 69). No relations were found between the TC haplotype and age at diagnosis, extraintestinal manifestations, IBD family history, need for surgery, and extent of disease (data not shown).

Multifactorial analysis

Using multifactorial binary logistic regression considering the weight centile as either less than or more than the 9th centile, disease location and behaviour as defined by the Vienna classification, family history of IBD, erythema nodosum, and

Table 7 Allele frequencies and carriage rates for variants in the IBD5 haplotype in patients with ulcerative colitis compared with healthy controls

	UC patients (n = 74) v healthy controls (n = 256)	p Value	Odds ratio
Allelic frequency			
IGR2096a_1	50.7% v 42.0%	0.07	1.42 (0.97 to 2.08)
IGR2198a_1	50.7% v 41.0%	0.04	1.48 (1.02 to 2.15)
IGR2230a_1	52.9% v 47.5%	0.25	1.24 (0.85 to 1.81)
OCTN1	52.1% v 42.9%	0.05	1.45 (1.00 to 2.10)
OCTN2	54.1% v 47.9%	0.18	1.28 (0.89 to 1.86)
Homozygous carriers			
IGR2096a_1	27.9% v 15.2%	0.01	2.16 (1.15 to 4.05)
IGR2198a_1	26.8% v 15.2%	0.04	1.92 (1.03 to 3.58)
IGR2230a_1	27.5% v 21.2%	0.26	1.41 (0.77 to 2.60)
TC Haplotype	28.2% v 16.1%	0.02	2.04 (1.10 to 3.78)
Combined IBD5 haplotype	26.6% v 14.7%	0.02	2.10 (1.09 to 4.05)

Three marker single nucleotide polymorphisms (SNPs) (IGR2096a_1, IGR2198a_1, and IGR2230a_1) were used to represent the IBD5 haplotype together with two SNPs in the OCTN1 and 2 (1672C→T and -207G→C, respectively). The SNP IGR2198a_1 was associated with susceptibility to ulcerative colitis. Homozygous carriage both of the SNPs IGR2096a_1 and IGR2198a_1 and of the TC and IBD5 haplotype was associated with susceptibility to ulcerative colitis. The results for heterozygous carriage all failed to achieve significance (results not shown).
UC, ulcerative colitis.

Table 8 Comparison of patients and controls who are homozygous wild type for the IGR2198a_1 IBD5 haplotype marker single nucleotide polymorphism but have a risk allele in the TC haplotype

	Subjects with no IBD5 risk alleles	Subjects with TC haplotype but no IBD5 risk alleles
Controls	82	10
IBD patients	72	5
χ^2 p value comparing IBD patients with controls		0.27
CD patients	47	4
χ^2 p value comparing CD patients with controls		0.57
UC patients	18	0
χ^2 p value comparing UC patients with controls		0.20

The TC haplotype does not show an independent effect on disease susceptibility of the three IBD5 marker SNPs IGR2096a_1, IGR2198a_1, and IGR2230a_1, examined either individually or when combined in patients with IBD, Crohn's disease, or ulcerative colitis compared with controls. The data shown are only for IGR2198a_1, but results for all SNPs were non-significant and are available on request. CD, Crohn's disease; IBD, inflammatory bowel disease; SNP, single nucleotide polymorphism; UC, ulcerative colitis.

gastric antral Crohn's disease, a low weight centile was predicted by carriage of the TC haplotype ($p = 0.02$; OR = 3.41 (1.20 to 9.66)) and the presence of antral Crohn's disease ($p = 0.003$; OR = 4.02 (1.58 to 10.21)).

Using a logistic regression model to compare propensity across all 11 weight centile groupings, a very strong trend towards higher carriage of the TC haplotype at lower weight centiles and lower TC haplotype carriage at higher weight centiles ($p = 0.05$) was apparent at Crohn's disease diagnosis. Homozygous carriers of the extended IBD5 risk haplotype (patients who were homozygous mutants for all three IBD5 haplotype markers, IGR2096a_1, IGR2198a_1, and IGR2230a_1) were more likely to lie within lower BMI centiles and less likely to be within the highest BMI centiles in a logistic regression model ($p = 0.04$).

DISCUSSION

Our data provide a series of novel and important insights into the contribution of the IBD5 locus. We confirm a role in determining susceptibility to early onset Crohn's disease and report the first evidence of association in early onset ulcerative colitis. In addition, we have presented the first consistent genotype-phenotype data suggesting that these variants influence growth in both Crohn's disease and ulcerative colitis. We have shown that the OCTN1/2 variants do not act independently of variants within this region in determining susceptibility, emphasising the complexity of the IBD5 contribution.

Perhaps the unique strengths of our study lie in the robust phenotypic data that accompany the genetic data and in the size of the early onset population studied. A strong association between the IBD5 haplotype (and TC haplotype) with low weight and body mass index at diagnosis of Crohn's disease is evident, with a less significant association with height centile. The relation was demonstrated on unifactorial and multifactorial analysis for weight centile (with the TC haplotype) and for homozygosity for the extended IBD5 haplotype with BMI. Although the relation was most marked in Crohn's disease, there was a strong suggestion that it was also present in ulcerative colitis. Given the relatively small numbers of patients with ulcerative colitis, the study may have been underpowered to assess this relation fully.

Our data represent perhaps the strongest to date relating genotype to abnormal growth variables in inflammatory

bowel disease. Previous paediatric studies have tried to correlate patient genotype with growth indices but overall results have been not been consistent between populations. One study has suggested variant alleles of the -238 promoter polymorphism in the TNF α gene protect patients from height retardation.¹⁰ Tomer *et al* showed a relation between carriage of NOD2/CARD15 variant alleles and weight below the 5th centile (but not for height; BMI was not examined) and no follow up data were presented.¹¹ The data in Tomer's study were not subjected to multifactorial analysis; the findings have been confounded by the strong relation between NOD2/CARD15 carriers with ileal disease, which in turn is associated with lower weight at diagnosis.³⁸ Two subsequent publications have failed to replicate these findings.^{9, 39}

In our own population, we have studied the relation of NOD2/CARD15 genotype with growth indices; no relation was found at diagnosis, while at follow up carriers of NOD2/CARD15 variant alleles were more common in a low centile band for height (2-9th) and for weight (<0.4th) only.¹³

There is no universally accepted definition of growth failure or good evidence of how it should be treated.⁴⁰ Growth failure nevertheless remains a common problem, especially in paediatric Crohn's disease, and can persist into adult life.⁴¹ There is also evidence from prospective paediatric studies that the growth failure is "preprogrammed", strongly suggesting a genetic influence.⁴² Although our data do not establish that the genotype predicts "growth failure"—because our strongest growth associations were with weight and BMI, not height—they do show that genetic markers have the potential to be used to predict growth problems, and even perhaps determine treatment strategies.

The association of markers in the IBD5 haplotype with low anthropometric centiles signifying a more severe disease phenotype are entirely consistent with our recent findings in Scottish adults with Crohn's disease.²⁶ Possession of the variant alleles of the TC haplotype in 374 Scottish adults with Crohn's disease was associated with disease progression at follow up and, on multifactorial analysis, the need for surgery—both phenotypic variables representing a severe disease phenotype. As discussed in an accompanying editorial to our paper,⁴³ other adult datasets may also implicate the IBD5 locus as a determinant of severe disease.

We describe a novel association of the TC haplotype with gastric antral Crohn's disease. Previous adult studies have linked possession of the TC haplotype with ileal,⁴⁴ colonic,²⁵ and perianal³³ Crohn's disease, but none of these studies considered a relation with Crohn's disease of the proximal gastrointestinal tract. The establishment of such a relation gives some justification for using a phenotypic classification of Crohn's disease location in genotype-phenotype studies that is more extensive than the Vienna classification—an issue that has been intensively examined by the 2005 Montreal World congress Working Party into the classification of IBD.²⁹ The relation of antral disease with low weight centile at diagnosis on multifactorial analysis is intriguing in the light of previous prospective paediatric studies establishing a link between jejunal Crohn's disease and low weight centile at diagnosis.⁸

Our data show an incidence of proximal gastrointestinal Crohn's disease that may appear high in comparison with published adult series,²⁶ but the figures are similar to the prospective paediatric Crohn's disease studies from Europe and North America.^{8, 45} The difference may in part reflect a different disease phenotype between paediatric and adult Crohn's disease, but may also reflect the different investigation practices of paediatric gastroenterologists who would regard routine upper gastrointestinal endoscopy at diagnosis as standard practice.³⁰

Table 9 Genotype-phenotype of carriage rates of the TC haplotype and homozygous TC haplotype in patients with Crohn's disease at the time of diagnosis

	TC haplotype variants carriage rates at diagnosis	p Value	Homozygous mutant TC haplotype carriage rates at diagnosis	p Value
Individual disease location*				
Oral	79.2% v 75.3%	0.68	20.8% v 18.1%	0.75
Oesophageal	83.3% v 75.3%	0.65	50% v 15.8%	0.06
Gastric antrum	86.4% v 71.7%	0.05	36.7% v 14.2%	0.006
Gastric body	82.1% v 75.2%	0.43	14.3% v 18.2%	0.78
Duodenal	86.9% v 74.6%	0.19	26.1% v 17.5%	0.33
Jejunal	78.6% v 76.8%	0.84	17.8% v 18.2%	0.96
Ileal	75.0% v 78.2%	0.65	23.0% v 18.2%	0.48
Caecal	75.2% v 79.3%	0.65	18.8% v 17.2%	0.84
Sigmoid	75.8% v 81.8%	0.46	22.6% v 18.2%	0.58
Rectum	77.6% v 73.7%	0.61	22.4% v 23.7%	0.86
Perianal†	78.9% v 74.0%	0.46	22.5% v 22.0%	0.93
Perianal‡	83.3% v 75.3%	0.59	27.8% v 22.2%	0.45
Weight <9th centile	87.9% v 67.3%	0.002	28.8% v 16.8%	0.07
Height <9th centile	84.1% v 68.4%	<0.05	25.0% v 21.4%	0.62
BMI <9th centile	79.6% v 61.1%	0.02	27.8% v 16.8%	0.11
Location according to the Vienna classification²⁸				
Colonic	77.8% v 75.9%	0.80	28.9% v 21.1%	0.28
Ileal	77.8% v 76.3%	0.92	44.4% v 21.9%	0.21
Ileo-colonic	67.6% v 78.4%	0.18	17.6% v 24.3%	0.40
Upper GI	78.3% v 74.7%	0.57	20.5% v 25.3%	0.45
None§	85.7% v 76.0%	0.55	14.3% v 23.4%	1
Behaviour according to the Vienna classification²⁸				
Inflammatory	74.8% v 83.9%	0.28	22.4% v 25.8%	0.68
Stricturing	83.3% v 76.2%	0.68	16.7% v 23.3%	1
Penetrating	84.0% v 75.2%	0.33	28.0% v 22.2%	0.52
Atopic disease				
Eczema	69.8% v 77.3%	0.27	15.9% v 26.1%	0.12
Asthma	73.1% v 75.4%	0.74	11.5% v 26.9%	0.02
Hayfever	72.5% v 75.9%	0.66	7.5% v 27%	0.009
All atopy	60.0% v 76.3%	0.16	0% v 25.3%	0.02
No atopy	75.6% v 74.5%	0.86	29.1% v 17.7%	0.07

The clinical features of the Crohn's disease patients included in the study comparing carriage of the TC and homozygous TC haplotype showing the percentage carriage of each phenotypic variable compared with the percentage carriage of those without the phenotypic variable. The odds ratios with 95% confidence intervals for significant results are given in the text.

*The presence or absence of evidence for Crohn's disease activity in each location was based on evidence from endoscopy, biopsy, or barium follow through and was independent of disease activity elsewhere in the gastrointestinal tract (see text).

†Perianal disease defined by the presence of fissures, perianal ulcers, abscesses, or fistulae but not by the presence of skin tags alone.

‡Perianal disease defined by the presence perianal ulcers, abscesses, or fistulae but not by the presence of skin tags or fissures.

§These were patients who fulfilled the diagnostic criteria for Crohn's disease but in whom the disease location did not fit with the Vienna classification.

BMI, body mass index.

The association between the IBD5 haplotype and susceptibility to Crohn's disease has now been clearly demonstrated in adult studies^{18–21 25 26} but here we present further data from an exclusive early onset population to replicate the findings in Rioux's genome-wide scan.¹⁴ Our data represent the first paediatric study in ulcerative colitis to date and complement adult data.¹⁸ It is noteworthy that the index study from Canada may have been underpowered to assess the association between IBD5 and ulcerative colitis, as it contained only 20 families with ulcerative colitis.¹⁴ Our own dataset is considerably larger than the index study; nevertheless is relatively small for a case-control association study, and therefore the data have to be interpreted carefully because of the small number of patients compared with controls. The TC haplotype has been associated with Crohn's disease susceptibility in three adult studies,^{24–26} but no previous association has been shown with ulcerative colitis. This difference may be explained by a greater influence of the TC haplotype in early onset disease, or may indeed reflect differing disease phenotypes in the paediatric and adult ulcerative colitis

populations, notably the higher proportion of paediatric patients with more extensive colitis.^{8 46}

Despite demonstrating an association between the OCTN1/2 variants and disease susceptibility, our data do not support these variants as independent predictors of disease when the IBD5 risk markers are considered. This finding is contrary to the findings of Peltekova's study,²⁴ but consistent with subsequent publications and presentations.^{25 26 47 48} These apparent inconsistencies are likely to reflect the haplotype block structure of the region as described by Daly *et al* (fig 1).¹⁶ The published studies to date, apart from our own studies in the Scottish IBD population, have only examined one haplotype marker, IGR2078a_1 (haplotype block 4), and explored its relation (or lack of relation) with the OCTN1/2 genes (haplotype block 7).^{24 25} Our study examined markers in block 4 (IGR2096a_1), block 5 (IGR2198a_1), and block 7 (IGR2230a_1), representing an extended haplotype across the region. Thus the apparently independent effect reported in Peltekova's data may reflect recombination events between haplotype block 4 and 7 in the Canadian population. This

issue would be best resolved by further studies in the Canadian dataset.

Given this uncertainty about the identity of the causal gene within the IBD5 locus it remains pertinent to examine the role of other genes within the region: interleukin 4,^{49–51} interleukin 13,⁵² and interleukins 3 and 5, together with GMCSF, are all plausible candidate genes within the linkage interval.^{53–54} The protective association of the homozygous TC haplotype with asthma, hayfever, and atopy would be consistent with the evidence from genome-wide scans that genes within the 5q31–33 region are not only important in IBD but also in asthma and atopy.^{55–58} Indeed, candidate genes within the 5q31–33 region, including IL12B gene⁵⁹ and IL13,⁶⁰ have been associated with asthma and atopy.

Conclusions

In summary, we have demonstrated evidence for genetic association between the OCTN1/2 variants within the IBD5 locus and susceptibility to inflammatory bowel disease, Crohn's disease, and ulcerative colitis. Of note, our study also describes novel and consistent phenotypic associations with low anthropometric centiles. However, the effect of the OCTN1/2 variants is not independent of the extended IBD5 region and further investigation of these and other candidate genes within the IBD5 linkage interval is necessary.

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EDITOR'S QUIZ: GI SNAPSHOT

An unusual cause of abdominal pain

Clinical presentation

A 39 year old man presented with right iliac fossa pain. He had a 10 year history of intermittent abdominal pain, diarrhoea, rectal bleeding, and orogenital ulceration. Previous colonoscopy was normal but biopsies showed an early indeterminate colitis. A barium follow through was suggestive of a terminal ileal stricture and a labelled white cell scan showed increased uptake in the right iliac fossa. A diagnosis of ileal Crohn's was made and he was started on intravenous steroids.

His symptoms settled and he was discharged on oral prednisolone and mesalazine. He subsequently had a colonoscopy which reached into the terminal ileum and looked normal. A week later, the pain recurred in spite of high dose oral steroids. His abdomen was soft with no signs of peritonism. Inflammatory markers were normal and stool cultures were negative. He had a plain abdominal radiograph (fig 1).

Question

What does the x ray show?

See page 1137 for answer

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Figure 1 Abdominal radiograph.

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